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ION CHANNELS – MEMBRANE TRANSPORT – INTEGRATIVE PHYSIOLOGY

NH_4^+ secretion in inner medullary collecting duct in potassium deprivation: Role of colonic H^+-K^+-ATPase

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NH_4^+ secretion in inner medullary collecting duct in potassium deprivation: Role of colonic H^+-K^+-ATPase.

Background. In K^+ deprivation (KD), gastric (g) H^+-K^+-ATPase (HKA) is suppressed, whereas colonic (c) HKA is induced in the terminal inner medullary collecting duct (IMCD). We hypothesized that in KD, cHKA is induced and can mediate the secretion of NH_4^+.

Methods. Rats were sacrificed after 2, 3, 6, or 14 days on regular (NML) or K^+-free (KD) diet. mRNA expression of HKA isoforms in terminal inner medulla was examined and correlated with NH_4^+ secretion in perfused IMCD *in vitro*.

Results. Urinary NH_4^+ excretion increased after K^+-free diet for six days. In terminal inner medulla, cHKA expression was strongly induced, whereas gHKA expression was decreased. NH_4^+ secretion increased by 62% in KD ($JtNH_4^+$ 0.57 vs. 0.92 pmol/min/mm tubule length, $P < 0.001$). Ouabain (1 mM) in perfusate inhibited NH_4^+ secretion in KD by 45% ($P < 0.002$) but not in NML. At luminal pH 7.7, which inhibits NH_3 diffusion, NH_4^+ secretion in IMCD was 140% higher in KD (0.36 vs. 0.15, $P < 0.03$) and was sensitive to ouabain. ROMK-1 mRNA expression was induced in parallel with cHKA in inner medulla.

Conclusions. These data suggest that in KD, cHKA replaces gHKA and mediates enhanced secretion of NH_4^+ (and H^+) into the lumen facilitated by K^+ recycling through ROMK-1.

At least two isoforms of H^+-K^+-ATPase (HKA), colonic (c) and gastric (g), are expressed in the collecting duct (CD) cells [1, 2] and are presumed to mediate the exchange of intracellular H^+ for extracellular K^+ [3, 4]. Although gHKA is expressed constitutively and is responsible for the reabsorption of a majority of HCO_3^- in outer medullary (OM) and inner medullary (IM) CD, cHKA does not play any role in H^+ secretion or K^+ reabsorption under normal conditions [3, 4]. However, cHKA shows adaptive regulation in pathophysiologic conditions such as potassium depletion (KD) [2, 5–9], NaCl deficiency [10], and proximal renal tubular acidosis [11], suggesting an important role for this exchanger in K^+, HCO_3^-, and Na^+ (or Cl^-) reabsorption in disease states.

In KD, cHKA is up-regulated and mediates K^+-dependent, HCO_3^- reabsorption in OMCD and IMCD, whereas gHKA expression and activity are suppressed [8, 9]. The reason for this switch from gHKA to cHKA in KD animals is not clear because both isoforms appear to have the same mode of operation and stoichiometry. This raises the possibility that besides exchanging intracellular H^+ for luminal K^+, cHKA might also function in other transport modes.

In addition to enhanced HCO_3^- reabsorption, KD is also associated with increased renal NH_4^+ excretion [12]. NH_4^+ is generated in the proximal tubule cell, secreted into the lumen via Na^+/H^+ exchange [13], transported to the medullary thick ascending limb, where it is reabsorbed via apical Na-K-2Cl cotransport [14] and K^+/NH_4^+ antiport [15, 16], accumulated in the renal medulla, and secreted into the CD [17]. Recent studies have indicated that NH_4^+ transport across the basolateral membrane of IMCD cells may occur via an active transport of NH_4^+ rather than NH_3 diffusion [18]. NH_4^+ secretion across the apical membrane of normal OMCD and IMCD cells has been thought to be via NH_3 diffusion and subsequent trapping by H^+ rather than direct transport of NH_4^+ [17]. Thus, we entertained the possibility that (*a*) cHKA could function in both NH_4^+-K^+ exchange and H^+-K^+ exchange mode, whereas gHKA only functions in H^+-K^+ exchange mode, and (*b*) cHKA induction in IMCD could mediate the exchange of intracellular NH_4^+ with luminal K^+, leading to enhanced NH_4^+ secretion in KD.

METHODS

Animal model

Male Sprague-Dawley rats (125 to 150 g) were placed on a potassium-deficient diet (catalog #960189; ICN Biochemicals, Inc., Cleveland, OH) for up to 14 days; control rats (NML) ate normal rat chow (Harlan Teklad Co., Madison, WI, USA). Rats had free access to food and

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Table 1. Composition of solutions (in mM)

	Solution A	Solution B
Na^+	141	141
K^+	5	5
Ca^{2+}	1	1
Mg^{2+}	1.2	1.2
Cl^-	121	96
HCO_3^-	25	50
$H_2PO_4^{2-}$	1	1
SO_4^{2-}	1.2	1.2
Glucose	5.5	5.5
L-alanine	6	6
NH_4^+	5	5
pH	7.4	7.7

Inner medullary collecting duct (IMCD) lumen was perfused with either pH 7.4 (solution A) or pH 7.7 (solution B). For bath, only solution A (pH 7.4) was used.

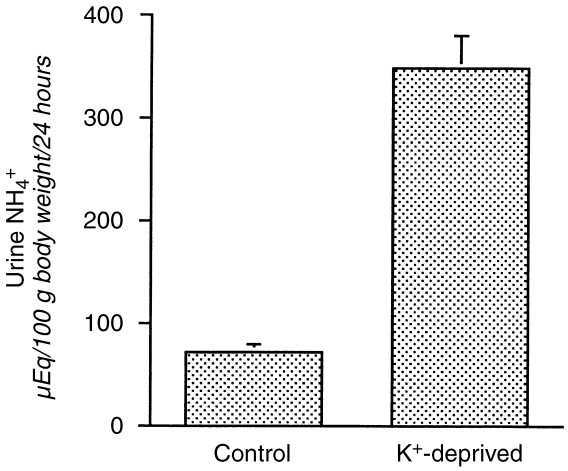

Fig. 1. Urinary NH_4^+ excretion in potassium depletion (KD). Twenty-four hour urinary [NH_4^+] was measured on day 6 ($P < 0.01$ vs. control).

water and were sacrificed by intraperitoneal injection of 50 mg of sodium pentobarbital. Intracardiac blood was obtained at sacrifice for plasma K^+ concentration measurement.

Urinary NH_4^+ excretion

Animals were housed in metabolic cages (NML, $N = 4$; KD, $N = 4$), and 24-hour urine samples were collected for volume and NH_4^+ determinations on day 6. Urinary NH_4^+ concentrations were measured by fluorometry (Nanoflow®; World Precision Instrument, Sarasota, FL, USA) using Ammonia Reagent Kit (171-UV; Sigma, St. Louis, MO, USA).

NH_4^+ transport in inner medullary collecting duct

After six to eight days on the test diet, kidneys were decapsulated after removal, sectioned into three to four cross-sections, and immediately placed in a Petri dish containing dissecting solution. The papillary tip was separated and transferred into a second Petri dish containing dissecting solution maintained at 14°C under a dissecting microscope. Segments of IMCD were dissected from the lower one third of papillary tip under ×120 magnification [8, 19] and were transferred to a Lucite chamber containing bathing solution initially at room temperature. The tubules were perfused as previously described [8, 9, 19, 20]. Perfusion rates were maintained at 1 to 2 nl/min. Solution A (Table 1) was used for experiments at luminal pH 7.4. HCO_3^--containing solutions were bubbled with 5% CO_2/95% O_2 gas. Both perfusate and bath solutions contained 5 mM NH_4^+ Cl^- (Table 1). The bath solution was kept at 7.4 except as noted (Table 1, solution A) and was replaced every 30 minutes; the bath pH was 7.44 ± 0.01 when monitored for 30 minutes after replacement. For NH_4^+ secretion measurements at alkaline luminal pH, tubules were perfused at pH 7.7 (Table 1, solution B). In all perfusions, the order of perfusion (with or without inhibitors) was random. After exposure to an inhibitor, the tubule was perfused with the test solution for 15 minutes prior to the onset of collection. Each tubule was used for three separate measurements: one in the absence of any inhibitor (control) and two in the presence of different inhibitors. Ouabain (OUA; 1 mM) was used in the perfusate to inhibit cHKA [12] and Schering 28080 (SCH; 10 μM) to inhibit gHKA [3, 4]. The perfusionist was blinded at random to the nature of the perfusate or the inhibitor. Collections were made at 30-minute intervals in a precalibrated constant-bore collection pipette. The collected samples were placed in a Petri dish under mineral oil.

Measurement of NH_4^+ flux

NH_4^+ in nanoliter samples from collectate and perfusate was measured with the use of Ammonia Reagent Kit (171-UV; Sigma) by microfluorometry (Nanoflow®; World Precision Instrument). The net flux of NH_4^+ across the tubule epithelium was calculated as

$$JtNH_4^+ = (C_0V_0 - C_1V_1)/L$$

where $JtNH_4^+$ is the net flux of NH_4^+ (pmol/min/mm tubule length). C_0 is the concentration of NH_4^+ in the perfusion fluid (pmol/nl). C_1 is the concentration of NH_4^+ in the collected fluid (pmol/nl). V_0 is the perfusion rate (nl/min). V_1 is the collection rate (nl/min; in the absence of vasopressin, $V_0 = V_1$), and L is the length of the tubule (mm) [19, 20].

RNA isolation

Total cellular RNA was extracted from the papilla by the method of Chomczynski and Sacchi [21]. In brief, 0.2 gm of tissue from several KD or NML animals were pooled and homogenized at room temperature in 10 ml Tri Reagent (Molecular Research Center Inc., Cincinnati, OH, USA). RNA was extracted by phenol/chloro-

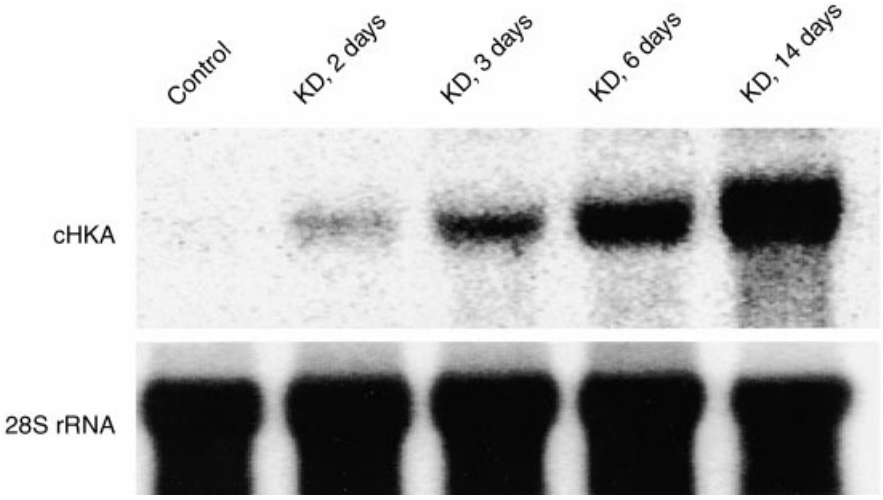

Fig. 2. Colonic H^+-K^+-ATPase (cHKA) Northern hybridizations in renal papilla of normal diet (NML; C) or potassium depleted (KD) rats. (*A*) cHKA Northern hybridization. (*B*) 28S rRNA Northern hybridization.

form, precipitated by isopropanol [21], quantitated by spectrophotometry, and stored at −80°C until it was used.

Northern hybridization

Total RNA samples (30 μg/lane) were fractionated on a 1.2% agarose-formaldehyde gel and transferred to Magna NT nylon membranes (MSI) using 10 × SSPE as transfer buffer. Membranes were then cross-linked by ultraviolet light and baked. Hybridization was performed according to Church and Gilbert [22]. The cDNA probes were labeled with ^{32}P-deoxynucleotides using the Rad-Prime DNA labeling kit (GIBCO BRL, Grand Island, NY, USA). The membranes were washed, blotted dry, and exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA, USA). For cHKA, three PCR products from the rat α subunit cDNA (nucleotides 135 to 515, 2369 to 2998, and 3098 to 3678) were pooled and used as isoform-specific probe. For gHKA, the EcoRV-PstI fragment from the α subunit was used as a specific probe. For renal outer medullary potassium channel (ROMK-1), the PCR product corresponding to nucleotides 38 to 611 from the corresponding rat cDNAs were used.

Materials

^{32}P-CTP was purchased from New England Nuclear (Boston, MA, USA). Nitrocellulose filters, agarose, ouabain (OUA), and other chemicals were purchased from Sigma Chemical Co. gHKA and ROMK-1 probes were generous gifts from Dr. Gary Shull and Dr. Nancy Baird, respectively, at the University of Cincinnati (Cincinnati, OH, USA).

Statistics

The data are expressed as mean ± SE where appropriate. For statistical analysis of mRNA expression experiments, the phosphor imager readings were obtained and analyzed. For functional studies, $JtNH_4^+$ was considered an approximation of net NH_4^+ secretion. Analysis of variance and *t*-test were used where appropriate to determine statistical significance. $P < 0.05$ was considered statistically significant.

RESULTS

Whole animal data

Serum $[K^+]$ in KD were 5.1 ± 0.4 mM at day 0, 5.2 ± 0.3 at day 2, 4.9 ± 0.4 at day 3, 4.3 ± 0.5 at day 6, and 2.9 ± 0.4 mM at day 14 ($P < 0.04$ for day 14 vs. day 0, $N = 4$ for each group).

Urinary NH_4^+ excretion increased ($P < 0.01$, $N = 4$ for NML or KD) by greater than fourfold in KD at day 6 (Fig. 1).

Northern hybridizations for HKA isoforms

By Northern hybridization, cHKA mRNA was induced in KD at day 2, and levels progressively increased at days 3, 6, and 14 (Fig. 2, upper panel). When adjusted for the level of expression of the constitutive control, 28S rRNA (Fig. 1, lower panel), cHKA mRNA levels in KD were increased ($P < 0.001$, $N = 3$ for each group) by 60-fold and 140-fold at 6 and 14 days, respectively.

NH_4^+ transport in inner medullary collecting duct

Net NH_4^+ secretion ($JtNH_4^+$) in IMCDt was higher in KD than in NML (−0.92 ± 0.04 vs. −0.56 ± 0.05 in NML pmol/min/mm tubule length; $P < 0.001$, $N = 6$ for NML and $N = 4$ for KD; Fig. 3A and Table 2).

In NML, $JtNH_4^+$ was not affected (P = NS) by OUA in the perfusate (−0.52 ± 0.06; Fig. 3B). SCH at 10 μM inhibited the NH_4^+ secretion minimally (the inhibition, however, did not achieve statistical significance). In contrast in KD, $JtNH_4^+$ decreased ($P < 0.002$, $N = 4$) in

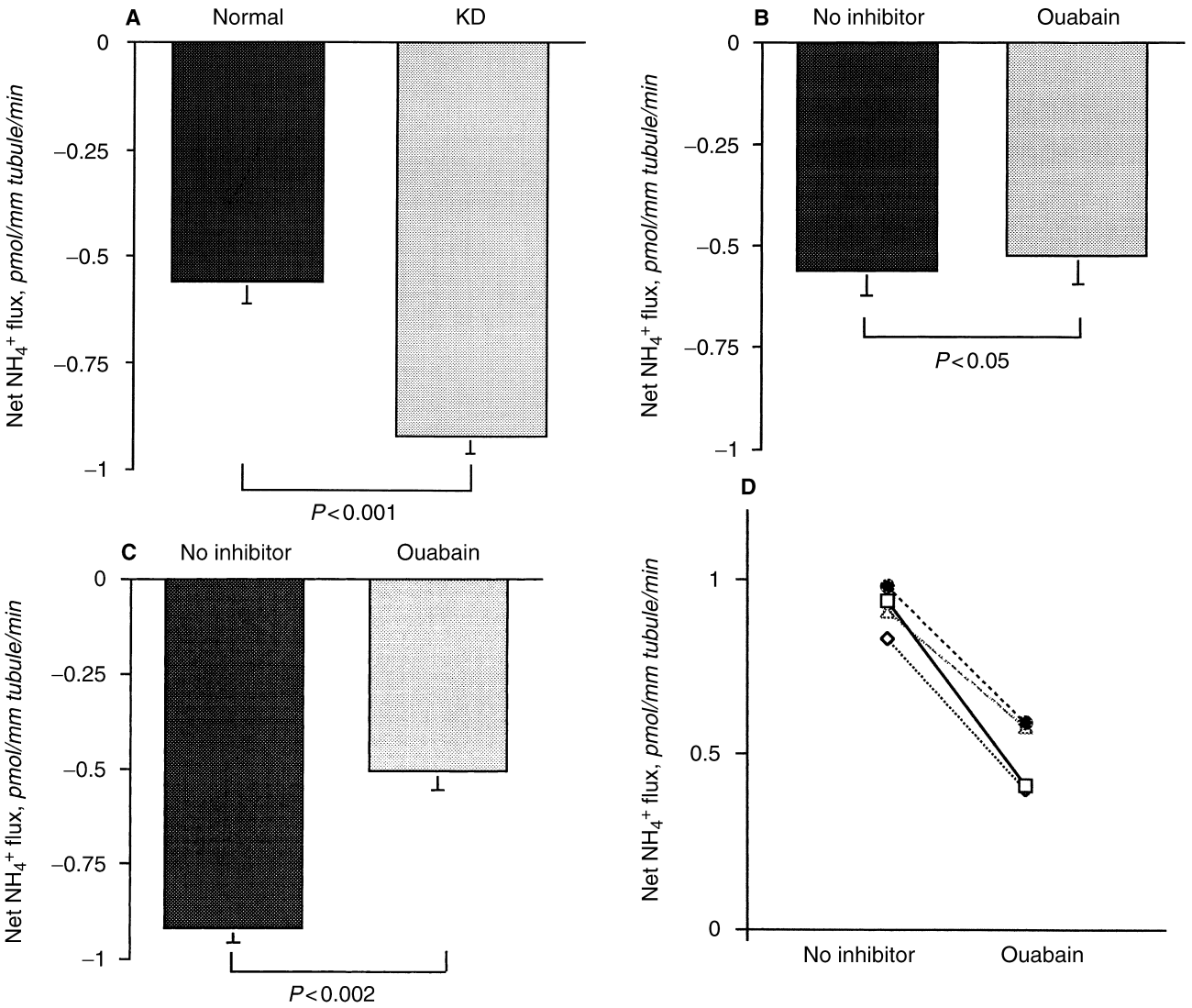

Fig. 3. (*A*) Net excretion of NH_4^+ ($JtNH_4^+$) in normal diet (NML; $N = 6$) or potassium depleted (KD; $N = 4$) rats. (*B*) Effect of 1 mM ouabain (OUA) in the perfusate on $JtNH_4^+$ in NML rats with no inhibitor ($N = 6$) or OUA ($N = 4$). (*c*) Effect of 1 mM OUA in perfusate on $JtNH_4^+$ in KD rats. Each bar represents mean ± SE for four separate experiments. The order of perfusion was random. Negative $JtNH_4^+$ values on the *Y* axis reflect secretion. (*C*) Individual flux rates from (c) animals are shown. The luminal and bath pH value for all animals was 7.4.

Table 2. Tubule lengths and perfusate and collectate NH_4^+ concentrations

Group inhibitor	Tubule length *mm*	Collectate NH_4^+ *mM*	Collectate NH_4^+		
			CON	OUA	SCH
NML[a]					
$N = 4$	0.99 ± 0.12	4.38 ± 0.29	5.04 ± 0.32	4.93 ± 0.30	4.89 ± 0.33
KD[a]					
$N = 4$	1.06 ± 0.15	5.08 ± 0.12	5.97 ± 0.09	5.47 ± 0.05	5.79 ± 0.09
NML[b]					
$N = 3$	1.60 ± 0.27	4.47 ± 0.06	4.72 ± 0.04	4.58 ± 0.03	4.60 ± 0.02
KD[b]					
$N = 4$	1.67 ± 0.16	4.17 ± 0.16	4.73 ± 0.13 CON	4.39 ± 0.14 K-free	4.64 ± 0.14 K-free + OUA
KD[a]					
$N = 6$	1.06 ± 0.10	4.34 ± 0.26	5.58 ± 0.44	4.96 ± 0.32	4.60 ± 0.34

Abbreviations are: NML, normal diet; KD, potassium deprived; CON, control; OUA, ouabain; SCH, Schering 280280.
[a] Perfusate pH = 7.4.
[b] Perfusate pH = 7.7.

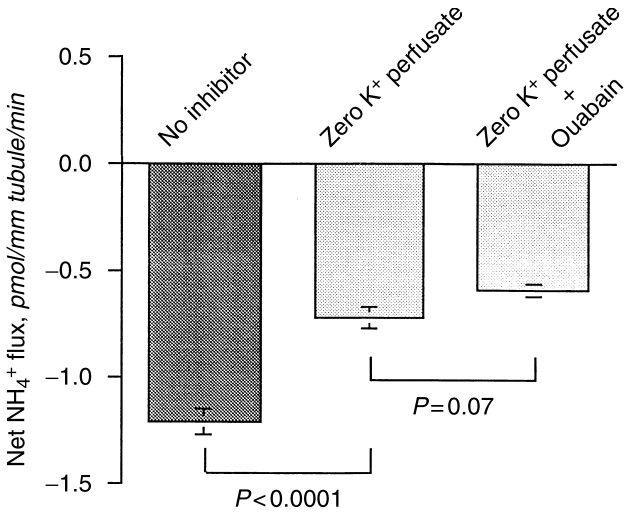

Fig. 4. Effect of perfusate K^+ on NH_4^+ secretion in potassium deprived (KD; $N = 6$) rats. IMCDt tubules were perfused with a normal or K-free solution at luminal pH 7.4. Negative $JtNH_4^+$ values on the Y axis reflect secretion.

the presence of OUA in perfusate to -0.50 ± 0.05 (Fig. 3 C, D) and decreased ($P < 0.03$, $N = 4$) in the presence of SCH in perfusate to -0.77 ± 0.03 pmol/min/mm.

The dependence of NH_4^+ secretion on luminal K^+ in KD was tested by replacing the Na^+ salts of solution A (Table 1) with K^+-containing salts. Removal of perfusate K^+ decreased ($P < 0.05$, $N = 6$) $JtNH_4^+$ from -1.21 ± 0.07 to -0.72 ± 0.06 (Fig. 4). Furthermore, the addition of OUA 1 mM to K^+-free perfusate did not inhibit (P = NS) $JtNH_4^+$ further (-0.72 ± 0.06 to -0.59 ± 0.03 pmol/min/mm; Fig. 4).

To determine whether NH_4^+ secretion is mediated by trapping of NH_3 by H^+, luminal pH was increased to 7.7 (Table 1, solution B) [17]. Alkaline luminal pH blunts the trapping of NH_3 by H^+ by increasing the back diffusion of NH_3, thereby decreasing net NH_4^+ secretion. In NML at luminal pH 7.7, $JtNH_4^+$ was decreased ($P < 0.01$) compared with that with a luminal pH of 7.4 (Figs. 5A vs. 3A) and was not inhibited further (P = NS, $N = 4$) by OUA (-0.15 ± 0.05 vs. -0.13 ± 0.04; Fig. 5B). In comparison in KD, $JtNH_4^+$ at luminal pH 7.7 was more than double that in NML (-0.36 ± 0.03 vs. -0.15 ± 0.05, $P < 0.03$, $N = 4$; Fig. 5A). This component of $JtNH_4^+$ decreased ($P < 0.002$, $N = 4$) from -0.36 ± 0.03 to -0.16 ± 0.03 pmol/min/mm in the presence of OUA (Fig. 5 C, D). SCH did not have any significant effect on $JtNH_4^+$ in NML or KD at luminal pH 7.7 (data not shown).

Northern hybridizations for ROMK-1

Because urinary K^+ excretion is reduced by more than 80% as early as 24 hours of KD (unpublished results) [23] before any up-regulation of cHKA, the functional operation of the induced cHKA is possibly linked to the activation or induction of a secretory K^+ mechanism. The low conductance K_{ATP} channel ROMK-1, which is normally expressed in OMCD but not IMCD, mediates K^+ secretion [24]. ROMK-1 mRNA levels were heavily induced in the terminal inner medulla in KD animals (Fig. 6). The expression of ROMK-1 was evident at 48 hours and progressively increased in parallel with cHKA. When adjusted for the level of 28S rRNA (membranes from Fig. 1 were stripped and used for ROMK-1 Northern hybridization), ROMK-1 expression increased by approximately 35-fold and 80-fold at day 6 and day 14 of KD, respectively ($P < 0.001$ for each group, $N = 3$).

DISCUSSION

These data show that urinary NH_4^+ excretion increases after six days of K^+-free diet, whereas serum [K^+] progressively declined, consistent with our previous studies [25, 26]. Contemporaneously, NH_4^+ secretion in the IMCD increased. In this setting, cHKA mRNA was heavily induced in the terminal inner medulla after 14 days of KD, whereas gHKA mRNA was diminished.

These data suggest that this increment in NH_4^+ secretion was mediated by cHKA. First, normal NH_4^+ secretion was inhibited by SCH and not by OUA, consistent with secretion mediated partially by gHKA as this response pattern to inhibitors is consistent with transport by the gHKA isoform [3, 4]. In contrast, the increased NH_4^+ secretion in KD was inhibited completely by OUA, a decrement of $JtNH_4^+$ of 0.42 pmol/min/mm. Although NH_4^+ secretion was also inhibited by SCH, albeit not in the magnitude seen with OUA, this pattern of inhibitor response in KD by cHKA has been seen previously by us and others [8, 9, 27]. The OUA-sensitive HKA in OMCD or IMCDt of KD rats shows sensitivity to SCH [8, 9, 27], which is distinct from the *in vitro* cHKA transfection experiments that only show sensitivity to OUA [28]. Based on the Northern hybridization experiments demonstrating down-regulation of gHKA [8] and the lack of additive effects of SCH and OUA on net HCO_3^- reabsorption in IMCDt of KD rats [8], we have suggested that the SCH-sensitive $JtNH_4^+$ at luminal pH 7.4 in KD animals likely reflects a contribution by cHKA and not gHKA. Second, a reduction in NH_4^+ secretion of similar magnitude was also induced by luminal K^+ removal and was not further inhibited by OUA. Taken together with the mRNA expressions, the data suggest that the increment in NH_4^+ secretion in KD was mediated by cHKA.

Our results suggest further that a major portion of the OUA-sensitive $JtNH_4^+$ in KD is due to the transport of NH_4^+ from cell to the lumen rather than trapping of NH_3 by H^+. Increasing the luminal pH from 7.4 to 7.7 inhibited $JtNH_4^+$ more in NML than in KD: $JtNH_4^+$ decreased from -0.57 to 0.15 in NML (approximately

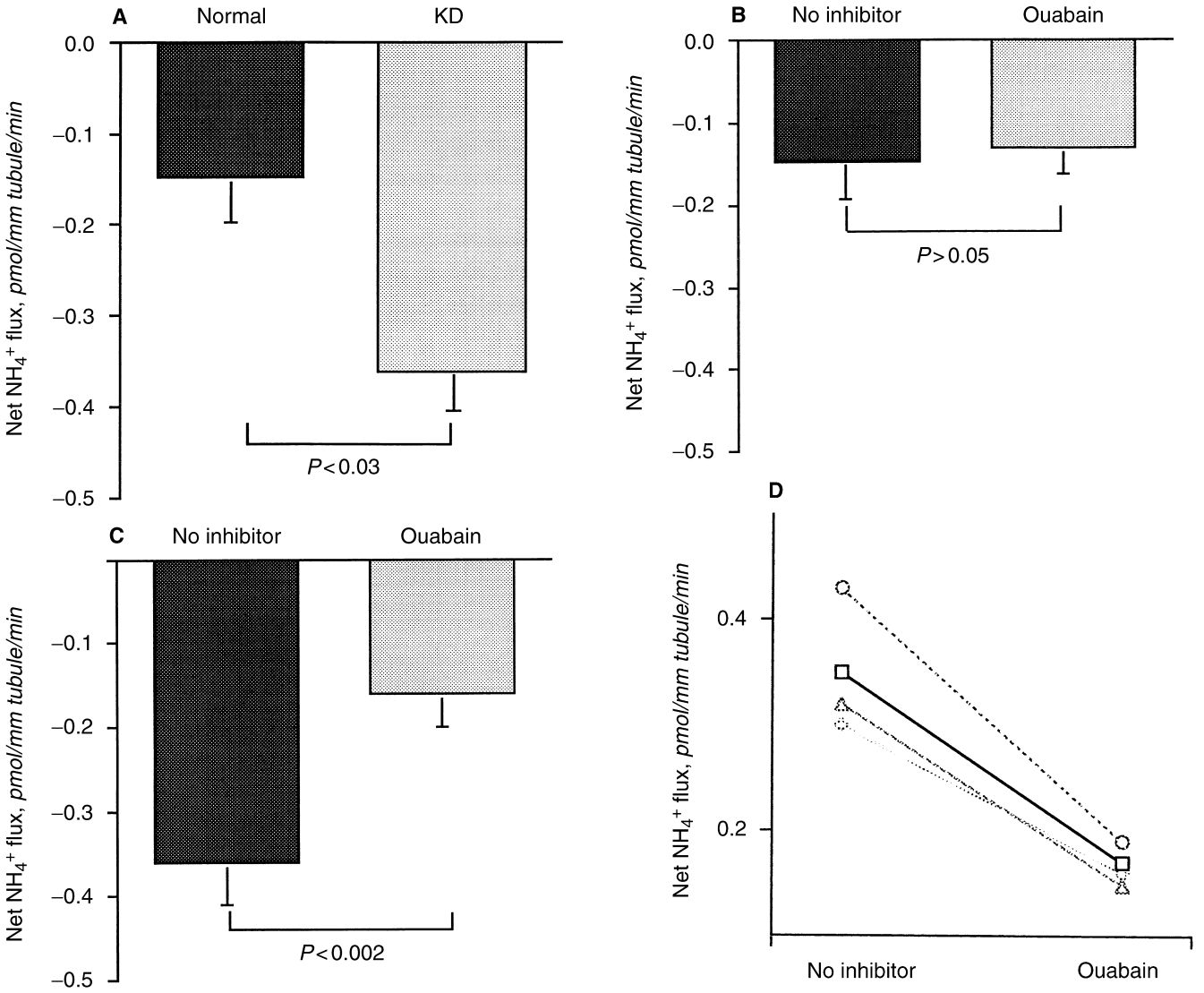

Fig. 5. (*A*) Effect of increasing luminal pH on $JtNH_4^+$ in the inner medullary collecting duct (IMCD) of normal diet (NML) and potassium deprived (KD) rats. (*B*) Effect of 1 mM ouabain (OUA) in perfusate on $JtNH_4^+$ in NML rats at luminal pH 7.7. (*C*) Effect of 1 mM OUA in perfusate on $JtNH_4^+$ in KD rats at luminal pH 7.7. Each bar represents mean ± SE for four separate experiments. Negative $JtNH_4^+$ values on the *Y* axis reflect secretion. The order of perfusion was random. (*D*) Individual flux rates from (c) are shown.

62% at luminal pH 7.4), whereas it decreased from −0.92 to −0.36 (approximately 140% at pH 7.7) in KD (Figs. 3A and 5A). When NH_3 diffusion was blunted by luminal alkalinization, the OUA-sensitive $JtNH_4^+$ of −0.19 completely accounted for the increment in NH_4^+ secretion in KD (Fig. 5 A, C). However, NH_3 trapping may also occur. To address this issue definitively, measurements of luminal and cellular pH would likely be needed to determine the NH_3 gradients.

Thus, we propose that cHKA can function in both (H^+)i-(K^+)o [8, 9] and (NH_4^+)i-(K^+)o exchange modes. In support of this, the OUA-sensitive net HCO_3^- reabsorption is increased in IMCDt in KD rats [8], consistent with the cHKA secreting H^+ into the lumen. It has also been shown that the H^+ binding site of the cHKA can accept Na^+ and function in (Na^+)i-(K^+)o exchange mode [29]. Thus, the internal H^+ binding site of cHKA appears to be a cationic binding site with affinity for H^+, NH_4^+, and Na^+. Whether other cations such as Li^+ can also interact with the internal cationic binding site of cHKA remains currently unknown.

It has been presumed that cHKA in KD is up-regulated to conserve K^+, as there is a need to minimize the urinary K^+ loss [2, 4, 5]. Parallel and simultaneous induction of cHKA and ROMK-1 suggests that reabsorbed K^+ is likely recycled back into the lumen via ROMK-1, suggesting that cHKA up-regulation in KD does not serve to conserve K^+ but rather to facilitate

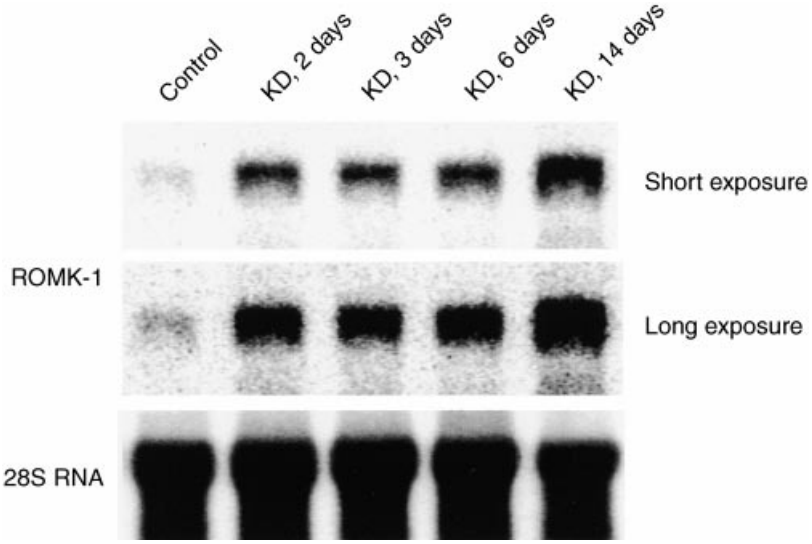

Fig. 6. ROMK-1 Northern hybridizations in renal papilla of normal diet (NML; C) or potassium deprived (KD) rats. Membranes from Figure 1 were stripped and probed with ROMK-1-specific cDNA.

the secretion of H^+ or an equivalent cation. It is in this context that ROMK-1 induction likely enables cHKA to function and exchange the luminal K^+ for an intracellular cation and that K^+ conservation occurs at some proximal site in the nephron.

In contrast to our results, down-regulation of ROMK mRNA in the medulla of KD rats has been shown [30]. However, the ROMK cDNA probe that was used in those studies was directed against the common domain of ROMK channels and could not distinguish among the three ROMK-1, ROMK-2, and ROMK-3 transporters. Furthermore, the entire medulla and not the papilla was sampled. Thus, it is possible that the decreased ROMK expression in KD in that study was due to down-regulation of ROMK-2, which is expressed in medullary thick ascending limb [30] and could have masked up-regulation of ROMK-1 in terminal inner medulla in KD.

Immunocytochemical studies with the use of Na^+,K^+-ATPase α-subunit–specific antibodies illustrate that in KD, the renal localization of this transporter remains restricted to the basolateral membrane domain, indicating that cell membrane polarity remains unchanged in this condition [31]. These studies further indicate that Na^+,K^+-ATPase expression in medullary CD is increased in KD [31]. Recent immunoprecipitation studies show also that the colonic H^+-K^+-ATPase α subunit coassembles with the Na^+,K^+-ATPase β subunit [32]. It is therefore possible that antibodies directed against the Na^+,K^+-ATPase β subunit may stain both the basolateral membrane as well as the luminal membrane. This latter property may actually be due to assembly of the β subunit with the colonic Na^+,K^+-ATPase α subunit.

Renal gHKA down-regulation and cHKA induction in KD is evident in both mouse (G.E. Shull, personal communication) and rat [8, 9, 23], indicating that this

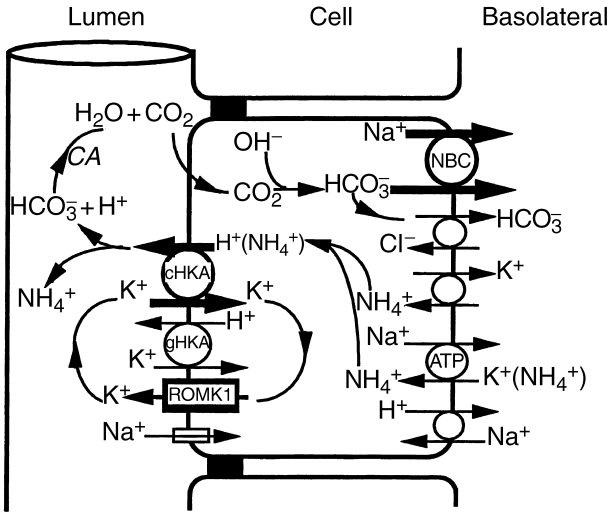

Fig. 7. Schematic diagram demonstrating adaptive regulation of H^+-K^+-ATPase (HKA) and other ion transporters in the inner medullary collecting duct (IMCD) of potassium deprived (KD) animals. The scheme shows induction of cHKA and ROMK-1 and up-regulation of NBC in KD (shown in dark lines). Basolateral Na^+-K^+-ATPase and K^+-NH_4^+ antiporter transport NH_4^+ into the cell. cHKA transports NH_4^+ into the lumen in exchange for K^+. ROMK-1 recycles the K^+ back into the lumen.

differential pattern is widespread among mammals. The reason for this switch from gHKA to cHKA in KD is not clear, as gHKA can also mediate H^+-K^+ exchange. We propose that one reason for this switch may be that cHKA can function in both (NH_4^+)i-(K^+)o and (H^+)i-(K^+)o exchange modes, whereas gHKA can only function in the (H^+)i-(K^+)o exchange mode. The schematic diagram (Fig. 7) illustrates the interaction of acid-base transporters in IMCD KD. According to this scheme, induction of the luminal cHKA and the basolateral

Na^+:$3HCO_3^-$ cotransporter NBC-1 (unpublished data) increases HCO_3^- reabsorption in KD. ROMK-1 provides the K^+ substrate for the operation of cHKA in secretion of NH_4^+ and H^+. In support of this hypothesis, addition of OUA to K^+-free perfusate did decrease NH_4^+ secretion further (although not significantly; Fig. 4), suggesting that secretion of K^+ into the lumen could have driven cHKA operation.

In conclusion, cHKA is induced in IMCD of KD rats together with ROMK-1. We suggest that cHKA can function in both (NH_4^+)i-(K^+)o and (H^+)i-(K^+)o exchange modes and mediate enhanced NH_4^+ secretion in KD facilitated by K^+ recycling via ROMK-1. cHKA might play an important role in the maintenance of metabolic alkalosis in KD by enhancing HCO_3^- reabsorption and NH_4^+ secretion in the medullary CD.

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